

**Supplementary material**

**Influence of genetic variability at the surfactant proteins A and D in community-acquired pneumonia: a prospective, observational, genetic study.**

M. Isabel García-Laorden, Felipe Rodríguez de Castro, MD, Jordi Solé-Violán, Olga Rajas, José Blanquer, Luis Borderías, Javier Aspa, M. Luisa Briones, Pedro Saavedra, J. Alberto Marcos-Ramos, Nereida González-Quevedo, Ithaisa Sologuren, Estefanía Herrera-Ramos, José M. Ferrer, Jordi Rello, Carlos Rodríguez-Gallego.

## METHODS

### Patients and controls.

Patients with defined severe immunosuppression or HIV positivity, as well as patients with tuberculosis, obstructive pneumonia caused by neoplasia or pneumonia as terminal event of chronic and progressive disease, were excluded from the study.

The pneumonia severity index (PSI) was measured using the Fine scale [17]. Acute respiratory distress syndrome (ARDS) was defined using the American European Consensus Conference Definition [18]. Multi-organ dysfunction syndrome (MODS) was defined using the American College of Chest Physicians/Society of Critical Care Medicine criteria [19]. A diagnosis of pneumococcal pneumonia was considered with one of the following criteria: 1) at least one blood, pleural fluid, or transthoracic needle aspiration culture positive for *S.pneumoniae*; 2) bacterial growth of  $\geq 10^3$  colony-forming units/milliliter (CFU/mL) of *S.pneumoniae* from a protected specimen brush, and/or  $\geq 10^4$  CFU/mL in bronchoalveolar lavage; 3) positive urinary antigen for *S.pneumoniae* with a diagnosis of probable pneumococcal pneumonia, using a commercially available immunochromatographic assay (Binax NOW).

### Statistical analysis

The comparison of the distribution of genotypes based on the susceptibility, severity and outcome were performed with the  $\chi^2$  test or Fisher exact test when needed, and odds ratios (OR) with 95% of confidence intervals (95% CI) were calculated. The relation between severity or outcome and genotypes was evaluated by binary logistic regression models, and age, gender, hospital of origin and co-morbidities, or PSI and pathogen were included as independent variables. Survival rates were estimated using the Kaplan-Meier method and their comparison related to genotypes was performed with log-rank test. Multivariate

analysis adjusted for the independent variables was carried out with Cox proportional hazard model. Alleles and haplotypes were recoded in binary variables for dominant and recessive effects. Quantitative variables are presented using arithmetic mean  $\pm$  SEM. The differences in serum levels with regard to the analyzed genotypes were compared with the U of Mann-Whitney, or with the H of Kruskal-Wallis when more than two genotypes were present. Statistical significance was taken as  $P$  value  $< 0.05$ . Bonferroni correction for multiple comparisons was applied when the frequencies of SNPs and haplotypes were compared between patients and controls.

## TABLES

**Table E1. Resulting haplotypes from SNPs combination in *SFTPA1* and *SFTPA2* genes.**

Haplotype	Nucleotide/amino acid				
<i>SFTPA1</i>	aa19	aa50	aa62*	aa133*	aa219
6A	C/Ala	C/Leu	G	G	C/Arg
6A <sup>2</sup>	T/Val	G/Val	A	A	C/Arg
6A <sup>3</sup>	T/Val	C/Leu	A	A	C/Arg
6A <sup>4</sup>	T/Val	C/Leu	G	A	T/Trp
<i>SFTPA2</i>	aa9	aa91	aa140*	aa223	
1A	C/Thr	C/Pro	C	C/Gln	
1A <sup>0</sup>	A/Asn	G/Ala	C	C/Gln	
1A <sup>1</sup>	C/Thr	G/Ala	T	A/Lys	
1A <sup>2</sup>	C/Thr	G/Ala	C	C/Gln	
1A <sup>3</sup>	A/Asn	G/Ala	T	A/Lys	
1A <sup>5</sup>	C/Thr	C/Pro	T	C/Gln	

SNPs: Single nucleotide polymorphisms.

Haplotypes are named as 6A<sup>n</sup> for *SFTPA1* and 1A<sup>n</sup> for *SFTPA2* based on previous nomenclature [15]. Only those haplotypes with a frequency higher than 1% are depicted.

\*Nucleotide change that does not produce amino acid change.

**Table E2. Demographic and clinical characteristics of CAP patients.**

Characteristics*	N (%)
Age (682)	62.95 ± 17.73 <sup>†</sup>
Sex (682)	
Male	435 (63.78)
Female	247 (36.22)
ICU admission (682)	
No (91.57±34.45 ) <sup>‡</sup>	526 (77.13)
Yes (118.41±40.29 ) <sup>‡</sup>	156 (22.87)
MODS (682)	
No (92.59±34.33) <sup>‡</sup>	593 (86.95)
Yes (133.68±39.86) <sup>‡</sup>	89 (13.05)
ARDS (682)	
No (96.67±37.23) <sup>‡</sup>	656 (96.19)
Yes (122.48±37.67) <sup>‡</sup>	26 (3.81)
90-day exitus (682)	
No (95.03±36.44) <sup>‡</sup>	636 (93.26)
Yes (134.67±33.60) <sup>‡</sup>	46 (6.74)
28-day exitus (682)	
No (95.70±36.66) <sup>‡</sup>	648 (95.01)
Yes (136.71±34.16) <sup>‡</sup>	34 (4.99)
Co-morbidity (670) <sup>§</sup>	
No	226 (33.14)
COPD	201 (30.00)
Asthma	29 (4.33)
Neoplasia	76 (11.34)
Ischemic cardiopathy	87 (12.99)
Diabetes	148 (22.09)
Renal insufficiency	50 (7.46)
Hepatic insufficiency	45 (6.72)
Neurological pathology	90 (13.43)
Autoimmune pathology	13 (1.94)
Psychiatric pathology	5 (0.75)
Pneumonia severity index (667)	
I-III (low)	290 (43.48)
IV-V (moderate-high)	377 (56.52)
Etiological agent identified (682)	
No	408 (59.82)
Yes	274 (40.18)
<i>Streptococcus pneumonia</i>	163 (23.90)
<i>Pseudomonas aeruginosa</i>	19 (2.79)
<i>Legionella ssp</i>	13 (1.91)
<i>Haemophilus influenza</i>	9 (1.32)
Virus	11 (1.61)
Others	59 (8.65)

CAP: Community-acquired pneumonia; ICU:

Intensive care unit; MODS: Multi-organ dysfunction

syndrome; ARDS: Acute respiratory distress

syndrome; COPD: Chronic obstructive pulmonary  
disease.

\*In brackets the number of patients with available data.

†For age the value is mean  $\pm$  standard deviation.

‡Pneumonia Severity Index (mean  $\pm$  SD).

§Some patients had more than one co-morbidity.

**Table E3. Pairwise linkage disequilibrium measure (D') for surfactant proteins****A1, A2 and D alleles from 748 healthy controls.**

	<i>SFTPA1</i> *							<b>SP-D</b>
	<b>6A</b>	<b>6A<sup>2</sup></b>	<b>6A<sup>3</sup></b>	<b>6A<sup>4</sup></b>	<b>6A<sup>5</sup></b>	<b>6A<sup>12</sup></b>	<b>6A<sup>15</sup></b>	
<b>1A</b>	0.34 (<0.0001)	0.72 (<0.0001)	-	-	1 (<0.0001)	-	0.65 (<0.0001)	0.31 (<0.0001)
<b>1A<sup>0</sup></b>	0.74 (<0.0001)	0.40 (<0.0001)	0.40 (<0.0001)	0.61 (<0.0001)	-	-	-	-
<b>1A<sup>1</sup></b>	-	0.50 (<0.0001)	0.51 (<0.0001)	-	-	-	-	-
<b>1A<sup>2</sup></b>	-	0.48 (<0.0001)	-	0.37 (<0.0001)	-	-	-	-
<b>1A<sup>3</sup></b>	-	-	-	-	-	-	-	-
<b>1A<sup>7</sup></b>	-	-	-	-	-	-	-	-
<b>1A<sup>10</sup></b>	-	-	-	-	-	-	-	-
<b>1A<sup>13</sup></b>	-	-	-	-	-	-	-	-
<b>SP-D</b>	0.93 (<0.0001)	-	-	-	-	0.84 (0.006)	0.71 (0.038)	

The numbers are D' (*P* value). Those D' values lower than 0.3, or with a corresponding *P* value higher than 0.05 have not been considered.

\*Haplotypes for *SFTPA1* and *SFTPA2*, resulting from the different combinations of the three SNPs (Single nucleotide polymorphisms) studied at each gene, are denoted using the conventional nomenclature [15].

**Table E4. Comparison of haplotypes of *SFTPA1*, *SFTPA2* and *SFTPD* between patients with pneumococcal CAP and controls.**

Haplotype*	Controls N=1538	PCAP N=326	<i>P</i> <sup>†</sup> OR (95% CI)
<i>SFTPA1</i>			
6A <sup>2</sup> (TGC)	934 (60.7)	177 (54.3)	0.77 <sup>0.032</sup> (0.60-0.99)
<i>SFTPA2</i>			
1A <sup>0</sup> (AGC)	911 (59.2)	169 (51.8)	0.74 <sup>0.014</sup> (0.58-0.95)
1A <sup>10</sup> (CCA)	4 (0.3)	5 (1.5)	5.97 <sup>0.011<sup>‡</sup></sup> (1.28-30.23)
<i>SFTPA1-SFTPA2</i>			
6A <sup>2</sup> -1A <sup>0</sup>	802 (52.1)	147 (45.1)	0.75 <sup>0.022</sup> (0.59-0.96)
<i>SFTPC-SFTPA1-SFTPA2</i>			
C-6A <sup>3</sup> -1A	3 (0.2)	4 (1.2)	6.36 <sup>0.021<sup>‡</sup></sup> (1.07-43.53)

Frequency values are the number of chromosomes (%).

PCAP: Pneumococcal community-acquired pneumonia.

Only those haplotypes with significant differences between PCAP and healthy controls were included.

\*Haplotypes for *SFTPA1* and *SFTPA2*, resulting from the different combinations of the three SNPs (Single nucleotide polymorphisms) studied at each gene, are denoted using the conventional nomenclature [15].

<sup>†</sup>*P* value for the bivariate comparison.

<sup>‡</sup>*P* value by Fischer exact test.